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THE ACTION OF CONESSINE AND HOLARRHENINE, THE ALKALOIDS OF HOLARRHENA CONGOLENSIS, AND ALSO OF OXYCONESSINE

BY

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THE ACTION OF CONESSINE AND HOLARRHENINE THE ALKALOIDS OF HOLARRHENA CONGOLENSIS, AND ALSO OF OXYCONESSINE

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Conessine, an alkaloid occurring in certain species of holarrhena, has been the subject of a previous physiological examination by Keidel.[1] The experiments here described were undertaken on account of a report, received from Frère Just. Gillet, S.J., a missionary in the Belgian Congo, of a local anaesthetic effect on the mucous membrane of the mouth produced by chewing the leaves of holarrhena congolensis. Dr. F. L. Pyman extracted the alkaloidal constituent of a small specimen of the leaves and found that it produced the effect described. Later, from a consignment of the bark, he obtained a quantity of the alkaloidal substance from which he separated two alkaloids. The first was recognized as conessine, which has also been called wrightine and which Haines[2] obtained as long ago as 1858 from holarrhena antidysenterica; to the second, a related but hitherto undescribed alkaloid, he has given the name holarrhenine.

For the preparations which I have used I am indebted to Dr. Pyman, who has elsewhere described his own and previous chemical work on the alkaloid. He has also supplied me with oxyconessine, an artificial oxidation product, first obtained by Warnecke.[3] The formulae of these preparations were as follows:

Conessine dihydrobromide $C_{24}H_{40}N_2$, 2HBr, $\frac{1}{2}H_2O$.
Holarrhenine dihydrobromide $C_{24}H_{38}ON_2$, 2HBr, $3H_2O$.

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[1] Keidel 1878, Uber die physiol. Wirkung d. Conessin. Inaugural-Dissertation, Göttingen.

[2] Haines 1858, Trans. Med. Phys. Soc. Bombay, n. s. vol. iv, 28.

[3] Warnecke, Arch. Pharm., 26, 281.

Oxyconessine sulphate $C_{24}H_{40}O_2N_2$, H_2SO_4, $3\frac{1}{2}H_2O$.

Keidel's account of the physiological action of conessine is given in somewhat general terms, the experimental details being few. It may be summarised as follows:

He found that the alkaloid depressed the centres in the brain for conscious sensation and for the initiation of voluntary movements It diminished the activity of the respiratory centre and in large doses paralysed it, causing death. The activity of the vasomotor centre was also diminished. Reflexes tended to disappear, any apparent increase in reflex action he considered to be a secondary effect brought about by interference with the circulation. He states that the peripheral nerves are unaffected by the alkaloid and that the "musculo-motor" centre of the heart remains unchanged. Conessine increased peristalsis and induced contraction of the bladder; it had no effect on the secretions.

Keidel concludes that in general the behaviour of conessine is very similar to that of morphine and chloral hydrate in being primarily a narcotic which in small doses can be used therapeutically.

Oxyconessine has some interest in that Warnecke described the occurrence in the urine of dogs after administration of conessine, of a substance giving similar colour reactions to those obtained with it. Neither oxyconessine, nor holarrhenine, has previously been the subject of physiological investigation.

I. On the central nervous system

I am unable to confirm Keidel's statement that conessine has a marked narcotic action on mammals. Whereas he observed narcosis after injecting 25 mgm. into a cat, I have observed no trace of such an effect even after large doses. Indeed the effects of hypodermic injection of conessine in mammalia are trivial.

In search of narcotic action on the part of the alkaloid 150 mgm. of conessine were injected hypodermically into a small cat weighing 1200 grams. As no effect of any sort was seen, and as the supply of conessine was limited, it was decided to continue with guinea-pigs. In the case of three pigs of weights from 300 to 400 grams, no narcotic action of any sort was seen when a dose of 150 mgm. was given to each. A pig weighing 300 grams, however, which received a dose of 250 mgm. subcutaneously showed more positive effects in this direction. It became sleepy, and when disturbed ran sluggishly a few paces, only to rest again. After half an hour it became more lively and grew very excited. Tremors were seen, particularly in its legs. The tremors became more pronounced and gave way in a few minutes to convulsions with ensuing death.

Twenty milligrams conessine was injected into the ear-vein of a small rabbit weighing 1500 grams. No effect was seen a quarter of an hour after the injection. At this point 50 mgm. were injected intravenously, with the result that the rabbit died in a few seconds.

It is clear, therefore, that conessine has but little effect on the central nervous system of mammalia. The cause of death in the experiments just described appeared to be paralysis of the respiratory centre, due to the alkaloid, but in each case the dose was large. The primary general narcosis in the case of the guinea-pig was moreover of the most insignificant nature.

On the other hand in the case of the frog, conessine produces more obvious effects. Thus 8 mgm. injected into the dorsal lymph sac of a 20 gram frog caused it to become sluggish in the course of a few minutes, and gradually all reflexes were lost. In half an hour there was no response to mechanical stimulus, however great. The fore-legs were seen to be clasped tightly across the chest, the conessine having produced an effect very similar to that of nicotine. After some hours this stage passed off, and the limbs became loose; finally by the following morning the frog was normal again. The paralysis proved to be central in origin, for another frog in which the narcosis had been similarly produced always responded with a twitch of the gastrocnemius muscle to electrical stimulation of the corresponding sciatic nerve.

All that has been said with regard to the action of conessine on the central nervous system can be repeated for holarrhenine. In the case of a frog which received 10 mgm. into its lymph sac, there was seen the same anaesthesia, and the same clasping of the fore-legs across the chest. In guinea-pigs there was the same absence of narcotic effect.

II. Action on peripheral nerves

The local anaesthetic action of the holarrhena alkaloids was the starting point of this investigation, and of the two conessine possesses the action in a more marked degree than holarrhenine. The action has been tested in two ways: (a) by application to the cornea in watery solution, (b) by observing the blockage of motor impulses in a frog's nerve.

Results of the first method: A large rabbit was used. Into the right eye were put about four drops of a 5 per cent solution of conessine, and into the left four drops of a 5 per cent solution of cocaine. After 3 to 5 minutes it was impossible to elicit a corneal reflex from the right eye, even when a horn spatula was rubbed hard against the eye. The conessine produced no sign of irritation. The anaesthesia in the left eye followed about 2 minutes later, and in each case when tested by this method, conessine acted slightly more powerfully than cocaine.

Compared with holarrhenine, the conessine was again more powerful. Thus four drops of a 6.6 per cent solution of conessine produced complete anaesthesia in the right eye of a rabbit in 9 minutes. Complete anaesthesia from a holarrhenine solution of the same concentration simultaneously instilled into the left eye only followed in 20 minutes.

Results of the second method: A muscle-nerve chamber simplified from the one described by Adrian[4] was prepared. The sciatic gastrocnemius preparation from the frog was used. The chamber has three partitions; it receives the muscle in one; the main length of the nerve lies in the second chamber where the anaesthetic is applied; in the third partition the end of the nerve rests on two platinum electrodes.

The time taken for a known concentration of anaesthetic to stop conduction through a given length of nerve was then determined. The stimulus used was the same in all cases, being about 30 per cent above the maximal stimulus. This ensured that all the fibres in the nerve were being excited and at the same time was not so great a stimulus as might lead to current spread through the narcotised region. Care was taken to see that no temperature changes took place during the experiments.

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[4] Adrian 1912, Journ. Physiol., xlv, p. 400.

The general result was that by this method conessine was much less effective in stopping conduction than cocaine, while holarrhenine had practically no such power at all. Results were only held comparable when obtained on muscle nerve preparations from the same frog:

Temperature 14° C. Weight of frog 20 grams

PREPARATION	ANAESTHETIC	LENGTH OF NERVE	TIME TO STOP CONDUCTION
	per cent	*mm.*	*minutes*
From right leg	0.8, cocaine	27.5	7.0
From left leg	5.0, conessine	27.5	17.5

Weight of frog 20 grams

PREPARATION	ANAESTHETIC	LENGTH OF NERVE	TIME TO STOP CONDUCTION
From right leg	0.8, cocaine	27.5	7.0
From left leg	6.0, conessine	27.5	14.0

A 10 per cent solution of holarrhenine had no effect on conduction through a sciatic nerve in 30 minutes.

It was recently pointed out by Fromherz[5] that different local anaesthetics give somewhat widely different relative values when examined by these two methods. This fact is well illustrated here. By the eye method conessine is slightly stronger than cocaine, while by the muscle-nerve method it is almost ten times weaker. Similarly with holarrhenine. By the eye method it is at least comparable with conessine, though weaker, but by the second method it has no action at all. It is not clear what is the reason for these apparent discrepancies. Possibly conessine and holarrhenine have a specific effect on nerve endings as opposed to nerve fibres in continuity, while cocaine acts impartially on both. Possibly the effect of conessine and holarrhenine on the corneal

[5] Fromherz 1914, Arch. f. exp. Path. u. Pharm., lxxvi.

nerve endings is only part of a general irritant action they possess on all living tissue. This however would hardly seem to be the case, as no trace of irritation was visible in any of the rabbits' eyes either during the application, or afterwards when the eye had recovered.

III. *Action on the circulation*

When conessine is injected intravenously into a cat or dog either when anaesthetised by ether, or after destruction of the brain, a rise of blood-pressure is observed (fig. 1). The rise remains after destruction of the spinal cord, so that it cannot be due to a stimulation of the vaso-motor centres in the cord by the alkaloid. Similarly the rise of blood-pressure is still produced when so much nicotine has already been injected that further injection of nicotine is without effect; the conessine rise is therefore not due to a stimulation of sympathetic ganglia. As a rise is again obtained after excision of the suprarenals from the circulation, it cannot be due to a stimulation of these glands by the conessine. Finally a rise is obtained after ergotoxine, when the animal is in the condition in which adrenalin itself produces

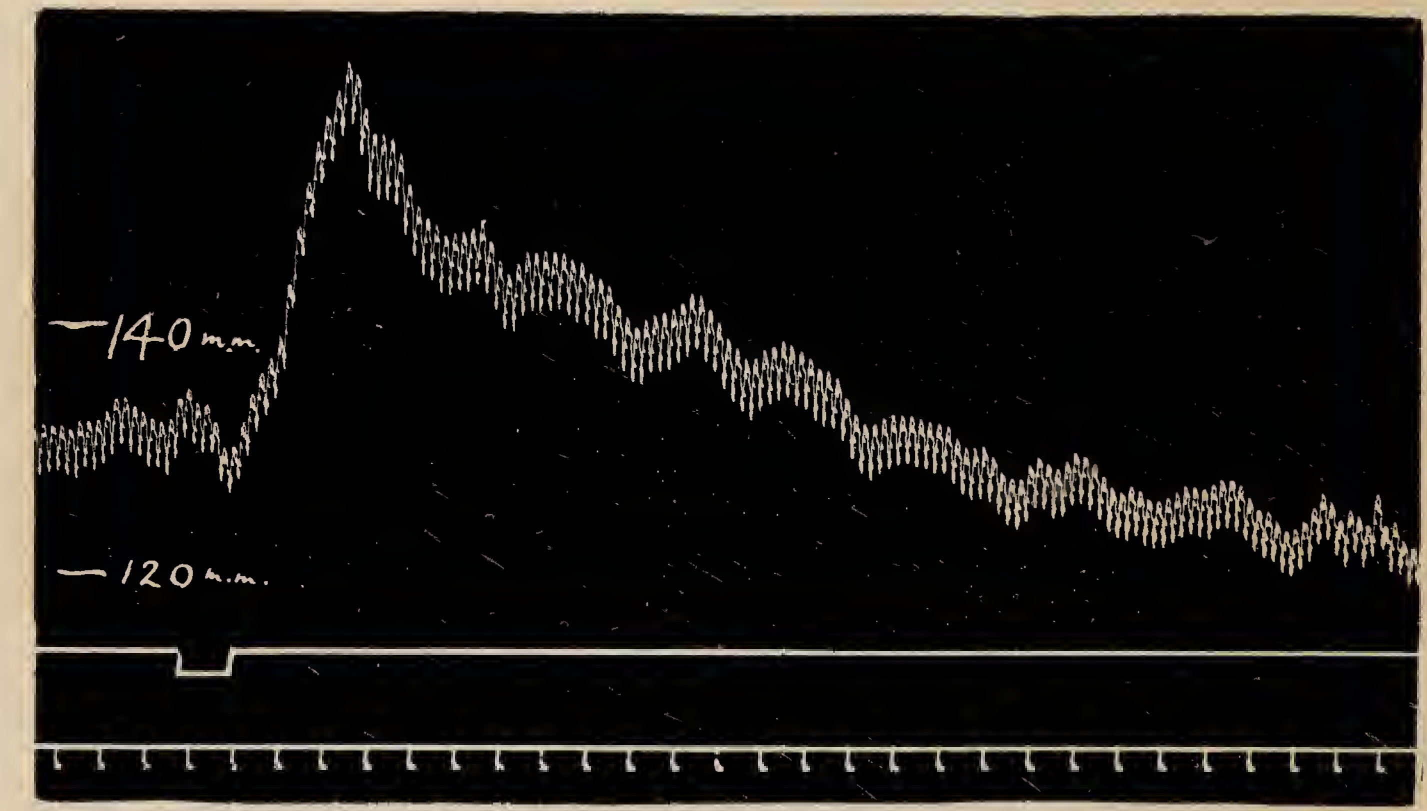

Fig. 1. Cat, 2000 grams. Ether. Carotid blood-pressure. 10 mgm. conessine dihydrobromide. Time in 10 seconds.

a fall of blood-pressure. An experiment may be described in detail in which this effect is seen:

January 28, cat ♀ 2500. Anaesthetised by chloroform followed by ether. Tracheotomy was performed and both vagi were cut. The brain was then destroyed, and a stiff wire with a hooked end was passed down the spinal cord several times. Bleeding from the brain and cord was stopped by plugging carefully with plasticine. At the moment of cutting the spinal cord artificial respiration was begun. A cannula for recording blood-pressure was then tied in the left carotid artery, and a second cannula in the right femoral vein for injection. The following injections were than made:

0.05 mgm. adrenalin. This produced a good rise of blood-pressure, which quickly passed off.

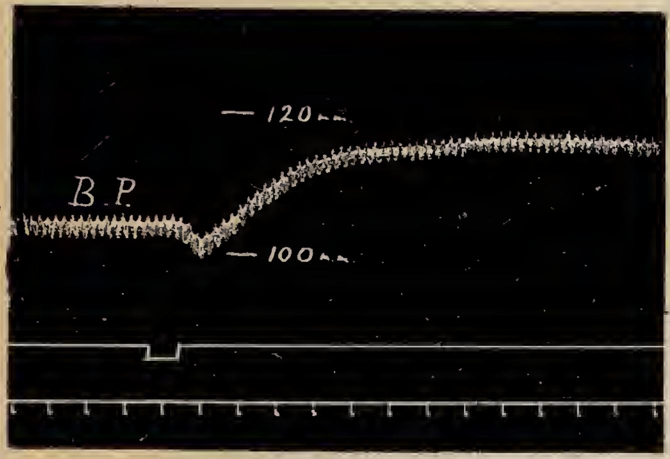

Fig. 2. Cat, 2400 grams. Brain and spinal cord destroyed. 4 mgm. ergotoxine previously given. 10 mgm. conessine injected at signal. Time in 10 seconds.

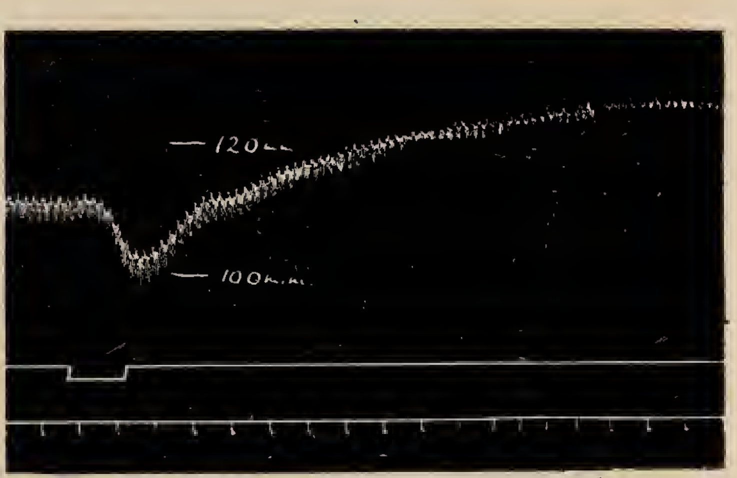

Fig. 3. Conditions as in figure 2. Injection 20 mgm. conessine at signal.

2 mgm. ergotoxine. Slow rise of blood-pressure more permanent.

2 mgm. ergotoxine. Slight further rise.

0.05 mgm. adrenalin. Fall of blood-pressure passing off to original level.

10 mgm. conessine. Slight momentary fall followed by rise of blood-pressure (fig. 2).

20 mgm. conessine. Larger initial fall followed by rise (fig. 3).

50 mgm. conessine. Large fall in blood-pressure. Heart became very irregular and remained so for three minutes, gradually however recovering and the blood-pressure returning to the former level. During the second minute 1 mgm. atropine was injected without any effect on the irregularity of the heart (fig. 4).

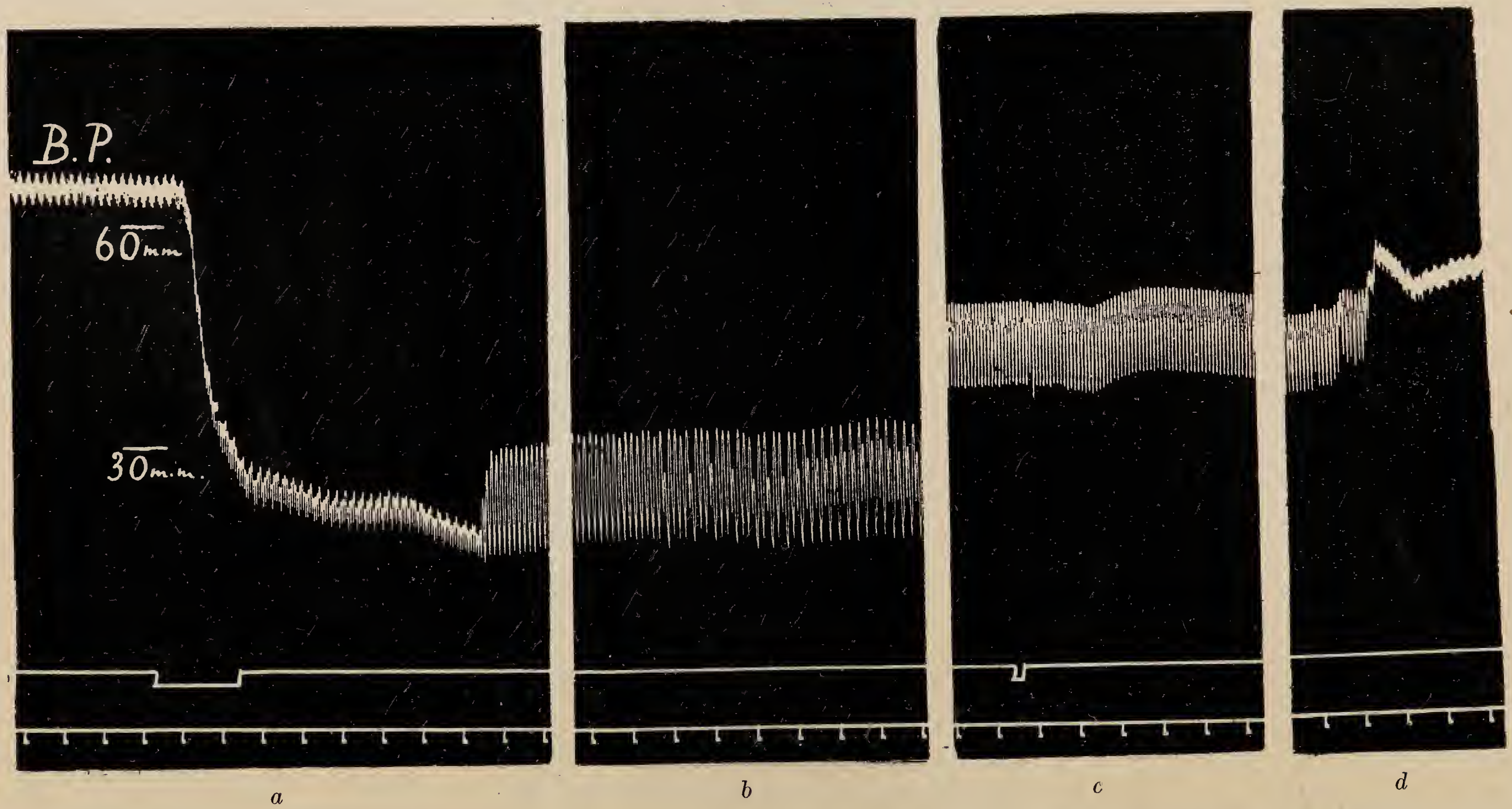

Fig. 4. Conditions as in figure 2. (*a*) Injection 50 mgm. conessine at signal; (*b*) 1 minute later; (*c*) 2 minutes later; 1 mgm. atropine injected at signal; (*e*) 3 minutes later. Heart recovers.

It is clear, therefore, that the rise of blood-pressure is due to a direct action of conessine on the muscle of the blood-vessel walls, and is, therefore, a true vaso-constriction.

An attempt was made to get further evidence of this vaso-constrictor action by perfusing the isolated rabbit's ear preparation described by Rischbieter[6] (originally introduced by Bissemski) with a Ringer's solution containing conessine. No change in the rate of flow, however, could be observed when a Ringer solution containing 1: 2000 conessine was substituted for Ringer alone.

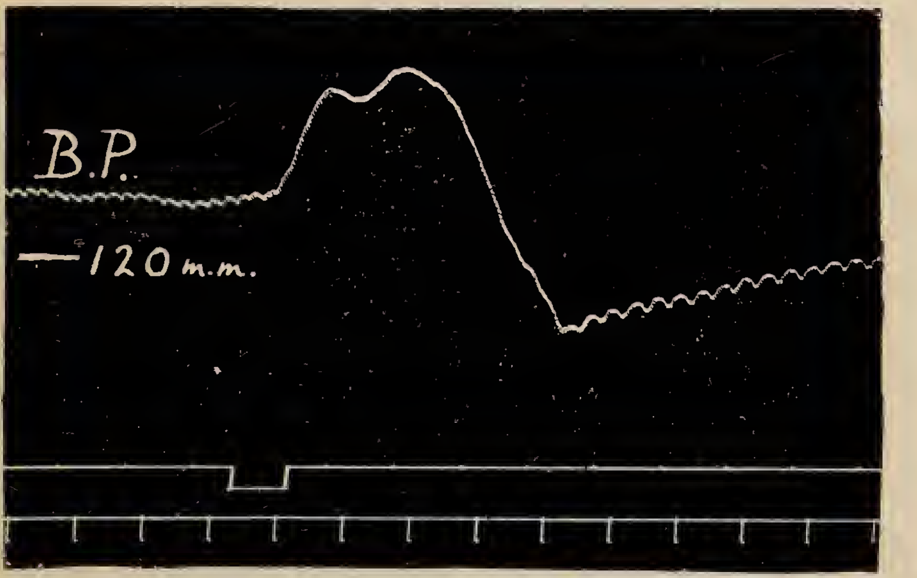

FIG. 5. Cat, 1700 grams. Ether. 10 mgm. holarrhenine dihydrobromide. Time in 10 seconds.

When injected intravenously holarrhenine gives exactly the same sort of results as conessine. A dose of 10 mgm. injected into the femoral vein of a cat breathing naturally under ether, produced a rise of blood-pressure accompanied by considerable slowing of the respiration (fig. 5). This rise was still produced in a cat to which a considerable dose of ergotoxine had been given, so that the rise is due to a true vaso-constrictor action as in the case of conessine.

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[6] Rischbieter 1913, Zeitschr. f. d. g. exp. Med., i, p. 356.

Action on the heart

In the experiment fully described above it will have been seen that a large dose of conessine produced a pure fall of blood-pressure of considerable extent, and that indications of this action were given in the principally pressor results of smaller doses. This fall is due to a weakening of the action of the heart by the alkaloid.

In order to examine this heart effect more closely, two perfusion experiments were performed on the isolated hearts of a cat and a rabbit, respectively. The phenomena observed were almost identical in the two cases, and it will be sufficient to describe the result in the case of the rabbit. A Locke's solution was used containing conessine in concentration 1: 50,000. After perfusion for half a minute the amplitude of the beats began to diminish steadily and after 1 minute the beats had disappeared altogether, leaving the heart quiescent between systole and diastole (fig. 6). With continued perfusion beats of small amplitude began again after 2 minutes, and then again passed off. Eight minutes later the tracing gave the appearance of regular beats of small amplitude; examination showed, however, that there was complete incoördination between auricles and

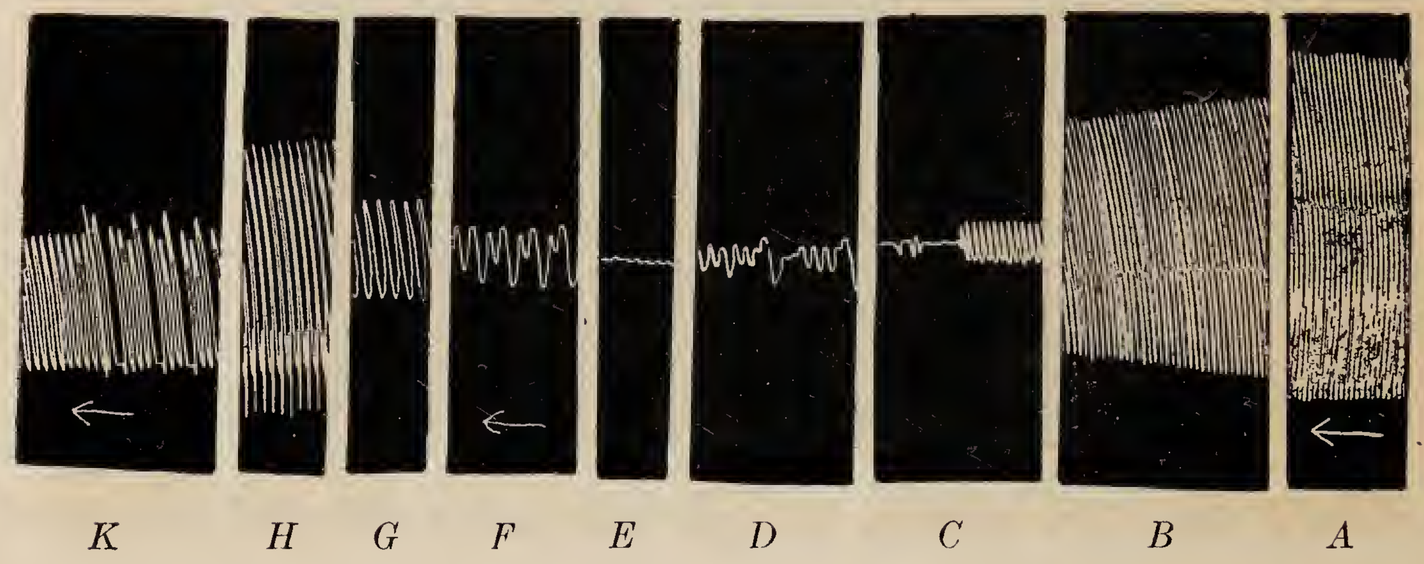

Fig. 6. Perfusion of rabbit heart with oxygenated Locke-Ringer solution. *A*, Normal; *B*, after change to 1: 50,000 conessine; *C*, 1 minute later; *D*, 2 minutes later than *C*; *E*, 2 minutes after *D*; *F*, 3 minutes after *E*; *G*, 4 minutes later than *F*; *H*, after perfusion of Locke-Ringer for 6 minutes; *K*, this shows the worst effect produced on the heart after a second perfusion of conessine.

ventricles. On perfusing once more with Locke's solution alone, this condition of incoördination, or block, gradually disappeared and after 6 minutes the beats were almost normal. During the course of the perfusion the rate of flow through the coronary circulation was not appreciably altered.

It was interesting to observe that a second perfusion of conessine had very little effect. The worst state following even prolonged perfusion for a second time was a partial block which soon passed off, leaving the heart beating regularly though with diminished amplitude.

A perfusion experiment on a rabbit's heart was also performed with holarrhenine. The effects were amost identical with those of conessine, with the difference that a solution of 1: 2000 holarrhenine did not act more powerfully than one of 1: 50,000 conessine. The results are seen in figure 7. There was the same gradual diminution in the size of the beats as with conessine, but prolonged perfusion did not lead to a return of the beat after this had once disappeared. The condition of heart block only supervened when the solution of holarrhenine had been washed out with normal Locke's fluid. With holarrhenine there was considerable constriction of the coronary flow.

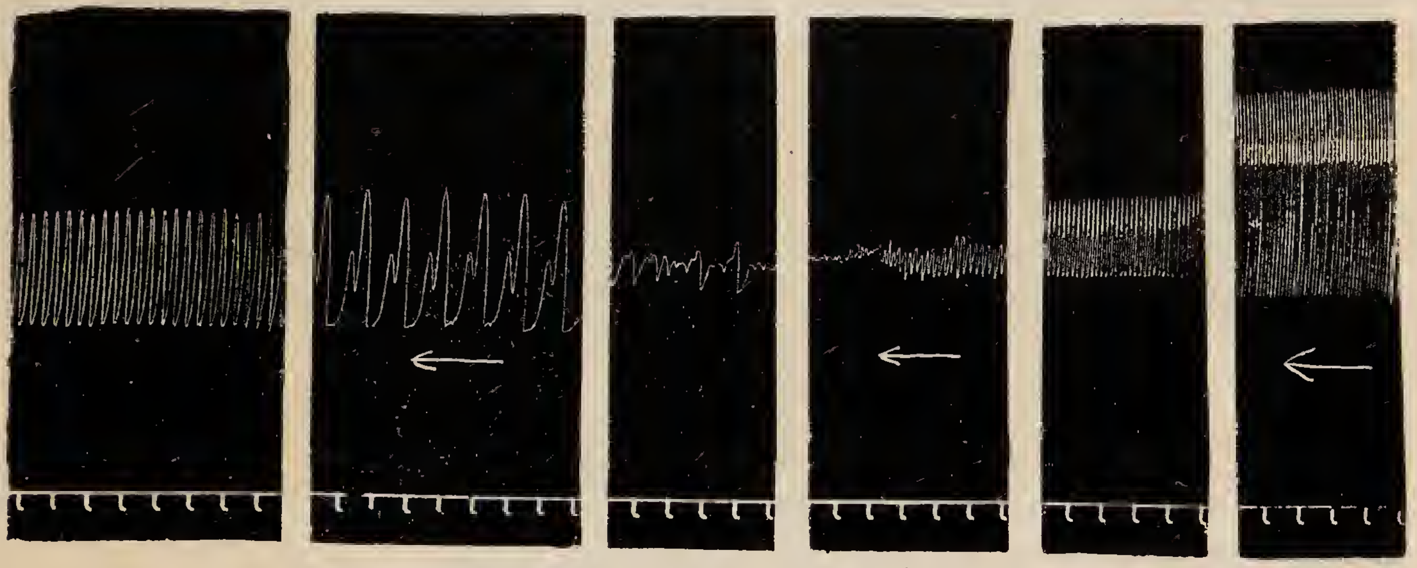

FIG. 7. Perfusion of rabbit heart with oxygenated Locke-Ringer solution containing 1: 2000 holarrhenine. Similar stages to those in figure 6. See text.

An initial rate of flow of 5 cc. in 13.4 seconds was changed after perfusion with the drug for half a minute to one of 5 cc. in 72 seconds.

Action on the frog heart

Similar changes were here observed in perfusion experiments with conessine. With 1: 10,000 solution in Ringer, there was a gradual increase of amplitude, suddenly giving way to complete incoördination between the auricles and ventricles. In about 15 seconds the ventricle took on a regular rhythm of its own, so that a record of the heart-beat from the tip of the ventricle gave but slight indication of the real state of affairs (fig. 8). A double record from the auricles and the ventricle showed that the former were beating three or four times to each ventricular beat (fig. 9).

With holarrhenine such an effect was only once obtained. In general the effect of perfusion was a gradual disappearance of the beat.

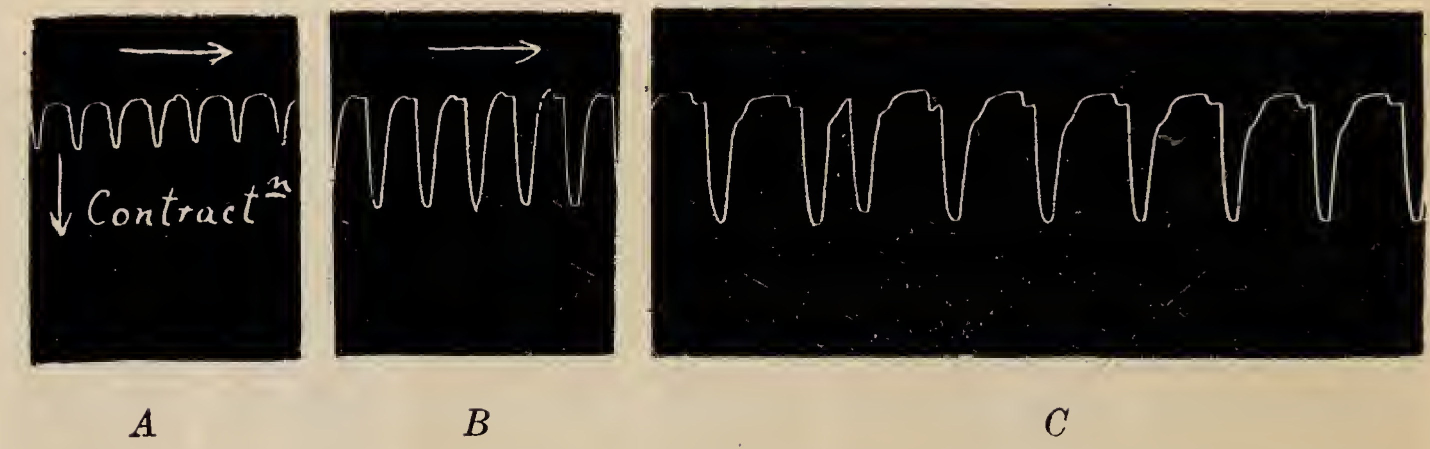

Fig. 8. Perfusion of frog heart with 1: 10,000 conessine. *A*, Normal; *B*, after perfusion for 1 minute with the alkaloid; *C*, stage of complete incoördination analysed in figure 9.

This capacity of the holarrhena alkaloids to produce auricular ventricular incoördination or "heart-block" is of some interest. The fact appears to be that the tissue of the *A–V* bundle is affected by contact with the alkaloids much more than is the tissue of the rest of the heart. Its conductivity is destroyed by them, whereas the rest of cardiac tissue can conduct perfectly well.

Action on plain muscle

With regard to other forms of plain muscle than blood vessels, conessine and holarrhenine are practically without effect. Five milligrams of the former added to a 50 cc. bath of Ringer at

37°C. produced a very small contraction of the isolated uterus of a virgin guinea-pig. Holarrhenine was without effect.

In the intact anaesthetised animal, conessine produced an ambiguous effect on the intestines. Records were taken with the Trendelenburg apparatus. In one case a marked contraction of the intestine was recorded with intravenous injection of 10 mgm. After nicotine paralysis on the other hand, there seemed to be an inhibition of intestine movement.

General toxicity

Comparatively large doses of these alkaloids may be given subcutaneously to cats, rabbits or guinea-pigs without any danger to the life of the animal. In every case of a dose given to a guinea-pig, however, even when the dose was so low as 10 mgm. it was found that two or three days after the injection there was considerable local necrosis and induration of the skin at the site of injection even when the whole procedure had been carried out with strict aseptic precautions.

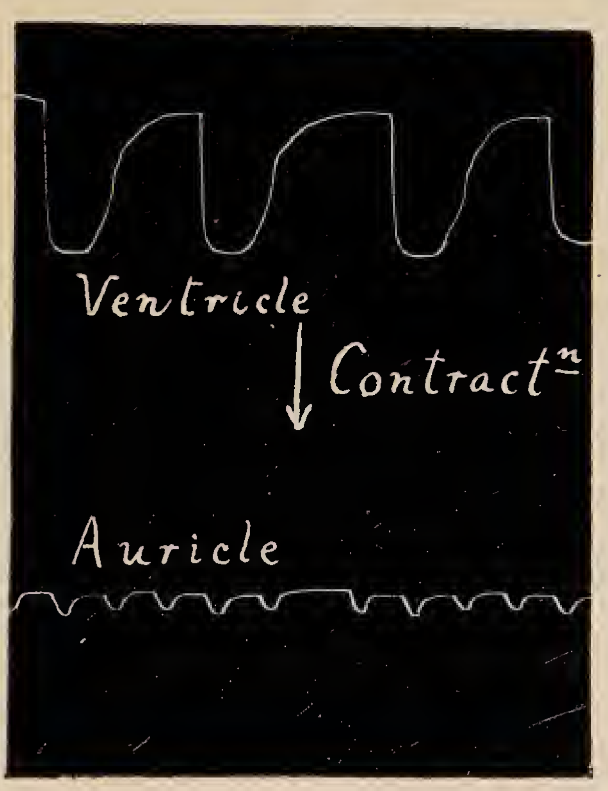

FIG. 9. Separate tracings from auricle and ventricle of frog heart seen in figure 8.

The smallest dose which, given subcutaneously, killed a guinea-pig was a dose of 250 mgm. conessine given to a pig weighing 300 grams.

WEIGHT OF GUINEA-PIG	DOSE	RESULT
grams	*mgm.*	
520	20	Lived
560	40	Lived
420	100	Lived
425	150	Lived
495	150	Lived
		(520–495) All developed large patches of necrotic tissue round point of injection
300	250	Died within 1 hour
320	500	Died within 1 hour

For frogs the results with conessine were as follows:

WEIGHT OF FROG	DOSE GIVEN IN 0.5 CC.	RESULT
grams	*mgm.*	
22	40	Dead within 1 hour
22	40	Dead within 1 hour
22	10	Dead within 3 hours
22	10	Dead within 3 hours
20	8	Recovered next morning
20	6	Recovered next morning

The results with holarrhenine on frogs were:

WEIGHT OF FROG	DOSE GIVEN IN 0.5 CC.	RESULT
grams	*mgm.*	
19	30	Dead within 3 hours
19	30	Dead within 3 hours
20	20	Dead within 3 hours
20	20	Dead within 3 hours
27	10	Just died within 5 hours
27	10	Just died within 5 hours

OXYCONESSINE

I. Action on the central nervous system

The result of subcutaneous injection into a frog appears to indicate at first sight that oxyconessine has a narcotic action on the frog at least as powerful as that of conessine. Almost immediately after the administration frogs lost all reflexes and remained in this condition until absence of heart beat showed that they were dead, or until they recovered. Further examination showed that this was a condition quite different from that of conessine narcosis. The paralysis was of the nature of a curare paralysis of motor nerve-endings, and was peripheral rather than central in origin. Half an hour after a dose of oxyconessine, it was impossible to elicit a contraction of the gastrocnemius by sciatic stimulation, though the muscle itself responded at once. Whether there was coincidently a central effect it was impossible to say. Subcutaneous injection of 100 mgm. had no effect on a small cat of 1500 grams.

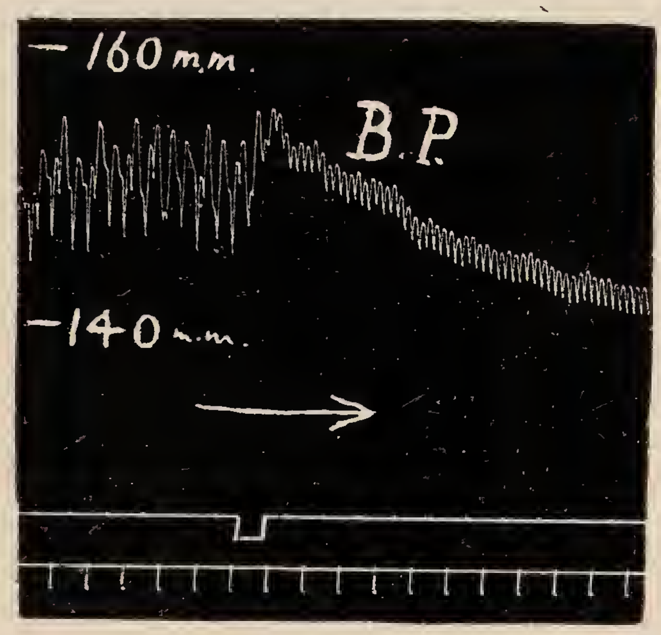

Fig. 10. Cat, 2000 grams. Ether. Blood-pressure tracing showing heart failure due to cessation of ordinary respiration. 7 mgm. oxyconessine injected at signal.

Oxyconessine is devoid of any local anaesthetic power. A 10 per cent solution applied to the cornea of a rabbit had no effect on the corneal reflex; similarly such a solution did not effect the conduction of impulses through the sciatic nerve of a frog.

Action on the circulation

If it is true that the body converts conessine into oxyconessine and excretes it in this form, as is suggested by the work of Warnecke, it would be expected that the physiological action of oxyconessine would differ from that of conessine in being less toxic. Such a difference does occur and it is most strikingly shown in the action of oxyconessine on the heart. When it was perfused through an isolated frog's heart, no change at all in the normal beat could be observed with a strength of 1: 5000. In fact so far from being harmful, it seems that oxyconessine has a beneficial effect, for an intravenous injection of 7 mgm. into a cat whose heart was failing due to asphyxia, steadied the heart immediately so that the beats became uniform and occurred at the normal rate (see fig. 10). Similarly when injected normally there was marked cardiac acceleration, with consequent rise of blood pressure.

Oxyconessine appears to have no vaso-constrictor action, and no power to cause contraction of plain muscle generally.

The lethal dose of oxyconessine

WEIGHT OF FROG	DOSE GIVEN IN 0.5 CC.	RESULT
grams	*mgm.*	
21	50	Curare effect in 15 minutes. Died
27	20	Curare effect in 15 minutes. Died
27	15	Curare effect in 15 minutes. Heart beating next day. Died
25	10	Curare effect in 15 minutes. Recovered
25	5	Curare effect in 15 minutes. Recovered
25	1	No effect

SUMMARY

The alkaloids of holarrhena congolensis, namely, conessine and holarrhenine, have a very similar physiological action, the former being more powerful than the latter. They both exert a considerable narcotic action on frogs, but in mammalia this effect is inappreciable. They also possess a local anaesthetic action particularly marked when tested on the rabbit's eye. This property is of no practical value, as both alkaloids produce local necrosis when injected subcutaneously.

Both conessine and holarrhenine are cardiac poisons, causing the isolated perfused heart to come to a standstill halfway be-

tween systole and diastole. They possess a particularly destructive action on the tissue of the *A-V* bundle, and even when injected intravenously produce incoördination, or "heart-block."

In small doses both alkaloids produce a rise in blood-pressure, when injected intravenously. This rise is still seen after paralysis of the vaso-constrictor nerve endings with ergotoxine, and must therefore be due to a direct action on the blood-vessel walls.

Oxyconessine, an artificial oxidation product of conessine, has somewhat different properties. It possesses a curare action on the frog, but appears to have no general or local anaesthetic action.

When injected intravenously it produces marked cardiac acceleration and consequent rise of blood-pressure. It has no true vaso-constrictor action.